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The synthesis of opiorphin and studies on its binding ability toward Cu(II)

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ABSTRACT

Opiorphin (OPI) is an endogenous pentapeptide isolated from human saliva. A characteristic feature of this peptide is the presence of an N-terminal Gln residue. The synthesis and binding abilities of opiorphin toward Cu(II) ions are described. The use of potentiometric and spectroscopic methods allows us to propose the binding mode of the formed complexes.

Opiorphin (OPI) is an endogenous pentapeptide isolated from human saliva[1] and belongs to the endogenous enkephalinase inhibitors (EIs) which possess antinociceptive, anticraving, antidiarrheal, and antidepressant activity.[1,2] As a result, opiorphin also influences colonic contraction[3] and plays a role in erective physiology.[4,5]

The structure of opiorphin showing the pentapeptide sequence H-Gln-Arg-Phe-Ser-Arg-OH is shown in Figure 1a.

The presence of an N-terminal glutamine in the opiorphin amino acid sequence makes this peptide interesting for studies on its interactions with metal ions. Literature reports on the coordination abilities of peptides with N-terminal Gln residues are few, however, there are reports of N-terminal Gln cyclization with the formation of the structure presented in Figure 1b.[6] Herein, we describe the synthesis of opiorphin, and based on electrochemical and spectroscopic studies, report on the interaction of OPI with copper(II) ions.

Opiorphin was synthesized using a solid-phase method on 2-chlorotrityl chloride resin (loading 1.3 mmol/g, 1% DVB, 100–200 mesh, Tianjin Nankai Hecheng Science and Technology Co., Ltd, China) using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.[7] The *N*-α-Fmoc-amino acids were protected on the side chain as follows: trityl (Trt) for glutamine, *tert*-butyl (*t*Bu) for serine, and 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) for arginine. The attachment of the first amino acid to the resin was performed according to Barlos et al. with a loading of 0.7 mmol per

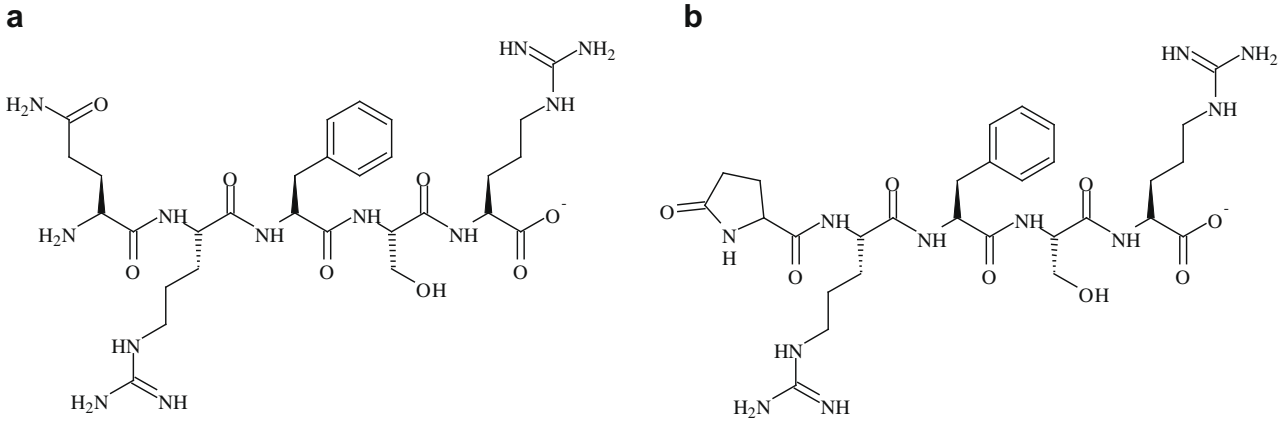

Figure 1. The structure of (a) opiorphin (Gln-Arg-Phe-Ser-Arg) and (b) its analogue with cyclic glutamine at the N-terminus (Glp-Arg-Phe-Ser-Arg).

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gram.[8] Deprotection of the Fmoc group was carried out over 5 and 15 min with 25% piperidine in *N,N*-dimethylformamide (DMF). All the amino acids, apart from Fmoc-Gln(Trt)-OH, were coupled using a three-fold molar excess of the protected amino acid (Fmoc-AA) dissolved in DMF and 1-hydroxybenzotriazole (HOBt) and diisopropylcarbodiimide (DIC) for 2 h (Fmoc-AA/HOBt/DIC = 1:1:1). The coupling reaction of Fmoc-Gln(Trt)-OH was carried out using *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) with the addition of HOBt in the presence of diisopropylethylamine (DIEA) for 2 h (Fmoc-AA/TBTU/HOBt/DIEA = 1:1:1:2). The extent of each coupling step was monitored by using the chloranil test. After the synthesis had been completed, the peptide was cleaved from the resin with a trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5 v/v/v) mixture for 2 h. The cleaved peptide was precipitated with diethyl ether and lyophilized. The crude peptide thus obtained was purified by reverse phase high performance liquid chromatography (RP-HPLC) on a Kromasil C8 column (8 × 250 mm, 5 μm particle size) with several linear gradients of acetonitrile in 0.1% trifluoroacetic acid (TFA). The eluates were fractionated and analyzed by analytical RP-HPLC. The purity of the peptide was determined on an analytical Beckman chromatograph with a Kromasil C8 column (4.6 × 250 mm, 5 μm particle size) with several linear gradients in 0.1% TFA. Fractions containing the pure peptide (>98%) were pooled and lyophilized. The peptide was analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF).

Analysis of the potentiometric and spectroscopic results[9] allowed us to characterize the coordination abilities of opiorphin.

Opiorphin has two protonation constants which can be assigned to the protonation of the N-terminal amino group and C-terminal carboxylate group (Table 1).

OPI has two arginine residues which are able to undergo dissociation. The literature shows that log *K* of the arginine residue is higher than 11.0 (>11.40)[10] and as the potentiometric titrations were performed up to pH 11, it was not possible to determine the protonation constants for both Arg residues.

The studied peptide forms five monomeric complexes: CuL, $CuH_{-1}L$, $CuH_{-2}L$, $CuH_{-3}L$, and $CuH_{-4}L$ (Table 2, Fig. 2). Coordination of copper(II) ions begins with binding of the metal ion to the nitrogen of the N-terminal amino group and formation of the CuL complex, however, because of the low concentration of this species (less than 10%), spectroscopic characterization was not possible.

Next, the $CuH_{-1}L$ complex is formed with the absorption maximum occurring at 668 nm. The EPR parameters (A_{II} = 170 [G], g_{II} = 2.252) support two nitrogen atoms bound to the copper(II) ion (Table 2). The appearance of two charge transfer bands at 312 and 270 nm suggests coordination of the nitrogens from the $-NH_2$ group and the first peptide bond. Above pH 6.5 the next two protons dissociate from the $CuH_{-1}L$ species and $CuH_{-2}L$ and $CuH_{-3}L$ appear in solution (Table 2, Fig. 2). The values of log *K* for

Table 1
Stability constants of the proton and copper(II) complexes of opiorphin

Species	log β	log *K*
H-Gln-Arg-Phe-Ser-Arg-OH		
HL	6.97 ± 0.01	6.97
H_2L	9.92 ± 0.01	2.95
CuL	3.93 ± 0.08	
$CuH_{-1}L$	−0.98 ± 0.01	4.91
$CuH_{-2}L$	−7.86 ± 0.02	6.88
$CuH_{-3}L$	−14.91 ± 0.01	7.05
$CuH_{-4}L$	−25.58 ± 0.02	10.67

$\beta_{i(\text{free ligand})} = [H_iL]/[H^+]^i[L]$; $\beta_{pqr} = [M_pH_qL_r]/[M]^p[H]^q[L]^r$; *i*, *p*, *q*, *r* = stoichiometric factors.

Table 2
Spectroscopic parameters for the Cu(II) complexes formed by OPI

Species	UV/vis		CD		EPR	
	λ (nm)	ε ($M^{-1}cm^{-1}$)	λ (nm)	$\Delta\varepsilon$ ($M^{-1}cm^{-1}$)	A_{II} (G)	g_{II}
H-Gln-Arg-Phe-Ser-Arg-OH						
$CuH_{-1}L$	668	63.4	528.1	−0.4499	170	2.252
			312	0.2001		
			274.6	−0.7777		
			251.9	−0.0904		
$CuH_{-3}L$	517	122.7	531.1	−0.8752	202	2.178
			309.5	0.2141		
			275.5	−1.1131		
			249.5	1.5891		
$CuH_{-4}L$	516	129.0	528.1	−0.8878	205	2.173
			311.1	0.2191		
			276.6	−1.1391		
			250	1.6348		

Ligand concentration = 1 × 10^{-3} mol dm^{-3}, ligand-to-metal ratio = 1.5:1.

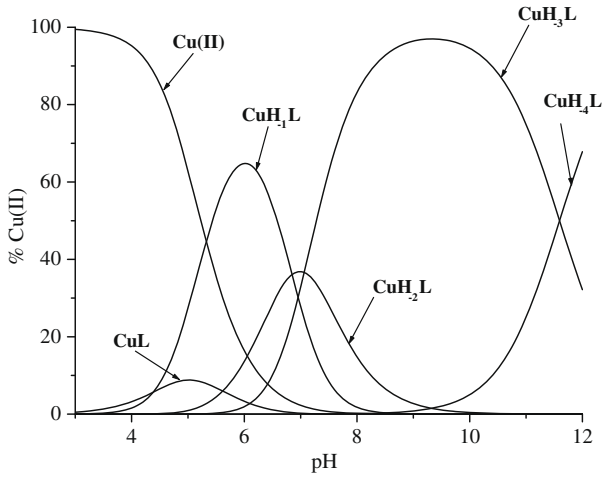

Figure 2. Species distribution curves for Cu(II)/OPI complexes at 25 °C, *I* = 0.1 mol dm^{-3} KNO_3. Ligand concentration = 1 × 10^{-3} mol dm^{-3}. Ligand-to-metal ratio = 1.5:1.

the $CuH_{-2}L$ (6.88) and $CuH_{-3}L$ (7.05) complexes strongly support proton dissociation from the second and third peptide bonds. The $CuH_{-2}L$ complex achieves the highest concentration—around 40% at pH 7 (Fig. 2). At the same pH two other species exist in solution, $CuH_{-1}L$ and $CuH_{-3}L$, with a concentration of about 30% for both and it was not possible to obtain the spectroscopic parameters for the $CuH_{-2}L$ complex. Above pH 7.5, the $CuH_{-3}L$ complex dominates in solution. The shift of λ_{max} 668 nm to 517 nm supports net binding of two nitrogens to the metal ion and formation of the 4 N type species. The presence of well-defined charge transfer in the CD spectra at 310, 276, and 250 nm shows binding of the $-NH_2$ and the peptide bond nitrogens (Table 2). Based on potentiometric and spectroscopic results we can assume that the $CuH_{-3}L$ complex is characterized by the {NH_2, N^-, N^-, N^-} binding mode.

Finally, the $CuH_{-4}L$ species appears in solution; $\log \beta_{CuH\text{-}3L} - \log \beta_{CuH\text{-}4L} = 10.67$ and any changes in the spectroscopic parameters for this complex support proton dissociation from one of the Arg moieties.[11]

Comparison of the coordination abilities of opiorphin and a simple pentapeptide such as Gly_5 shows that OPI is more effective in copper(II) binding (Figs. 3 and 4).

Opiorphin forms the same type of complexes as Gly_5, however, it is more effective in copper(II) ion binding. In comparison to Gly_5, OPI forms a more stable $CuH_{-1}L$ species (Fig. 3), which is probably

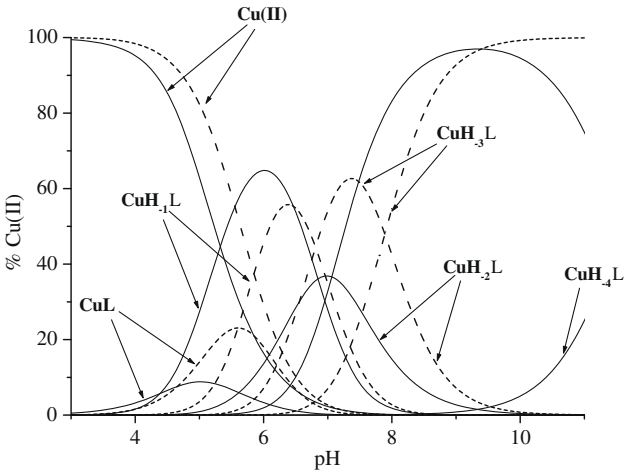

Figure 3. Species distribution curves for the complexes formed from Cu(II)/OPI (solid lines) and Cu(II)/Gly_5 (dashed lines)[12] as a function of pH.

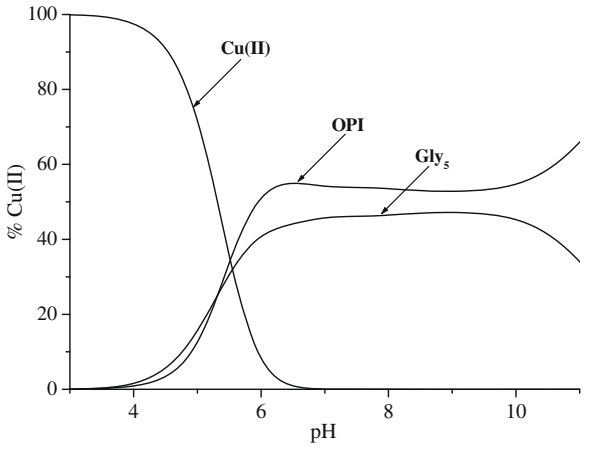

Figure 4. Competition diagrams for the OPI/Cu(II)/Gly_5 system.

caused by the presence of three side chains Gln-1, Arg-2 and Phe-3. As described above, $CuH_{-1}L$ is formed by the binding of two nitrogens (from Gln-1 and Arg-2). Formation of this species may result in some steric changes which additionally may induce an interaction between the side chains of Gln-1, Arg-2 and Phe-3. This interaction probably makes binding of the second amide nitrogen and formation of the $CuH_{-2}L$ complex more difficult in comparison with Gly_5. It may also have an influence by decreasing the stability for proton dissociation from one Arg moiety.

In conclusion, we have presented preliminary studies on the coordination ability of opiorphin with Cu(II) ions.

Acknowledgment

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References and notes

1. Wisner, A.; Bufor, E.; Messaoudi, M.; Nejdi, A.; Marcel, A.; Ungeheuer, M. N.; Rougeot, C. *Proc. Natl. Acad. Sci.* **2006**, *103*, 17979–17984.
2. Thanawala, V.; Kadam, V. J.; Ghosh, R. *Curr. Drug Targets* **2008**, *9*, 887–894.
3. Tian, X. Z.; Chen, J.; Xiong, W.; He, T.; Chen, Q. *Peptides* **2009**, *30*, 1348–1354.
4. Davies, K. P. *J. Sex Med.* **2009**, *6*, 286–291.
5. Tong, Y.; Tar, M.; Melman, A.; Davies, K.; Compilation, J. *Br. J. Urol.* **2008**, *102*, 736–740.
6. Orlowski, M.; Meister, A.. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: London, 1971; Vol. 4, pp 124–149.
7. Chan, W. C.; White, P. D. In *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Oxford University Press, 2000; pp 9–76.
8. Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Pept. Protein Res.* **1991**, *37*, 513–520.
9. The stability constants for the proton and Cu(II) complexes were calculated from titration curves recorded at 25 °C. NaOH (0.1 M) was added using a 0.250 ml micrometer syringe, which was calibrated by weight titrations of standard materials. The ligand concentration was 1×10^{-3} M and the metal-to-ligand ratios were 1:2. The potentiometric titrations were performed in 0.1 M KNO_3 (MOLSPIN pH-meter system) using a Russell CMAW 711 semimicro combined electrode (hydrogen ion concentration calibrated using HNO_3), and three titrations were performed on each system. A SUPERQUAD computer program[13] was used for stability constant calculations. Standard deviations were determined using SUPERQUAD and refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.
   Absorption spectra were recorded on a Perkin–Elmer Lambda Bio-20 spectrophotometer, circular dichroism (CD) spectra on a JASCO J 715 spectropolarimeter in the 200–850 nm range and electron paramagnetic resonance (EPR) spectra on a Bruker ESP 300E spectrometer at an X-band frequency of 9.4 GHz at 120 K. The ligand concentrations in these measurements were adjusted to 1×10^{-3} M and the metal-to-ligand ratios were 1:1.5. The spectroscopic parameters were obtained at the maximum concentration of the particular species.
10. Yamauchi, O.; Odani, A. *Pure Appl. Chem.* **1996**, *68*, 469–496.
11. Matera, A.; Brasuń, J.; Cebrat, M.; Świątek-Kozłowska, J. *Polyhedron* **2008**, *27*, 1539–1555.
12. Várnagy, K.; Szabó, J.; Sóvágó, V. *Dalton Trans.* **2000**, 467–472.
13. Gans, P.; Sabatini, A.; Vacca, A. *J. Chem. Soc., Dalton Trans.* **1985**, 1195–1200.